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LAYERED DIET FOR PINK BOLLWORM REARING

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ABSTRACT

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Discusses a method of rearing pink bollworms on layers of lima bean diet, alternately layered with waxed paper; improvements in methods and materials; and problems in rearing pink bollworms as a host for parasite production for large-scale field releases. This diet modification was used for rearing both the host and the egg-larval parasite, *Chelonus blackburni* Cameron. During four consecutive summers, over 4.6 million pink bollworm pupae were produced by this method, and 9.1 million *C. blackburni* were produced for field release.

KEYWORDS: pink bollworm, *Chelonus blackburni*; layered diet; parasite production; rearing insects.

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LAYERED DIET FOR PINK BOLLWORM REARING

By Raymond Patana[1]

INTRODUCTION

A laboratory culture of pink bollworms, *Pectinophora gossypiella* (Saunders), is maintained at the Cotton Insects Biological Control Laboratory, Tucson, Ariz., as a host for laboratory parasite and predator studies, and also as a host for parasites produced for field release. Patana[2] reported rearing the pink bollworm on lima bean diet using individual plastic cups and a bulk method using shredded diet. The individual cup method required excessive labor when large numbers of pink bollworms were needed, and moisture control in the shredded diet caused mold to develop. To overcome these problems, a somewhat different method of presenting the diet to the larvae was developed and has been in use for 6 years. This method consists of spreading the diet on sheets of waxed paper and then rolling the sheets of diet and paper together giving this combination a "jelly roll-like" appearance. The roll is then cut into sections, partially dried, and inserted into 3.8-liter cartons. The alternate layers of diet and paper offer some isolation for individual larvae, thus reducing cannibalism.

METHODS AND PROCEDURES

The hot (55° C) diet is spread in layers on 0.46-m wide waxed paper with a smooth paint roller to a thickness of about 0.3 cm.[3] The diet is spread on top of successive 1.7-m lengths of paper for a total of 12 layers (fig. 1). Origially, the diet was spread on a flat table surface, but when the successive layers were added, the diet tended to squeeze out from the edges, leaving them with less diet than the center. It was also difficult to aline the papers on top of each other when spreading the diet on a table surface. To overcome these problems, special racks were made. Each rack consists of a sheet of plastic 1.7 by 4.6 m by 0.5 cm attached to a wooden frame constructed from 7-cm-wide strips. The plastic is attached to the bottom of the frame to form an 0.45-m channel the length of the plastic with 2.54 cm of the side strips projecting above the surface of the plastic. The sides are held together by five cross strips under the

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[1] Research entomologist, Cotton Insects Biological Control Laboratory, Tucson, Ariz.

[2] Patana, Raymond. Rearing cotton insects in the laboratory. U.S. Dept. Agr. Prod. Res. Rpt. 108, 6 pp. 1969.

[3] Patana, Raymond, and McAda, W. C. Tobacco budworms: Use of dry diet flakes in rearing. Jour. Econ. Ent. 66: 817-818. 1973.

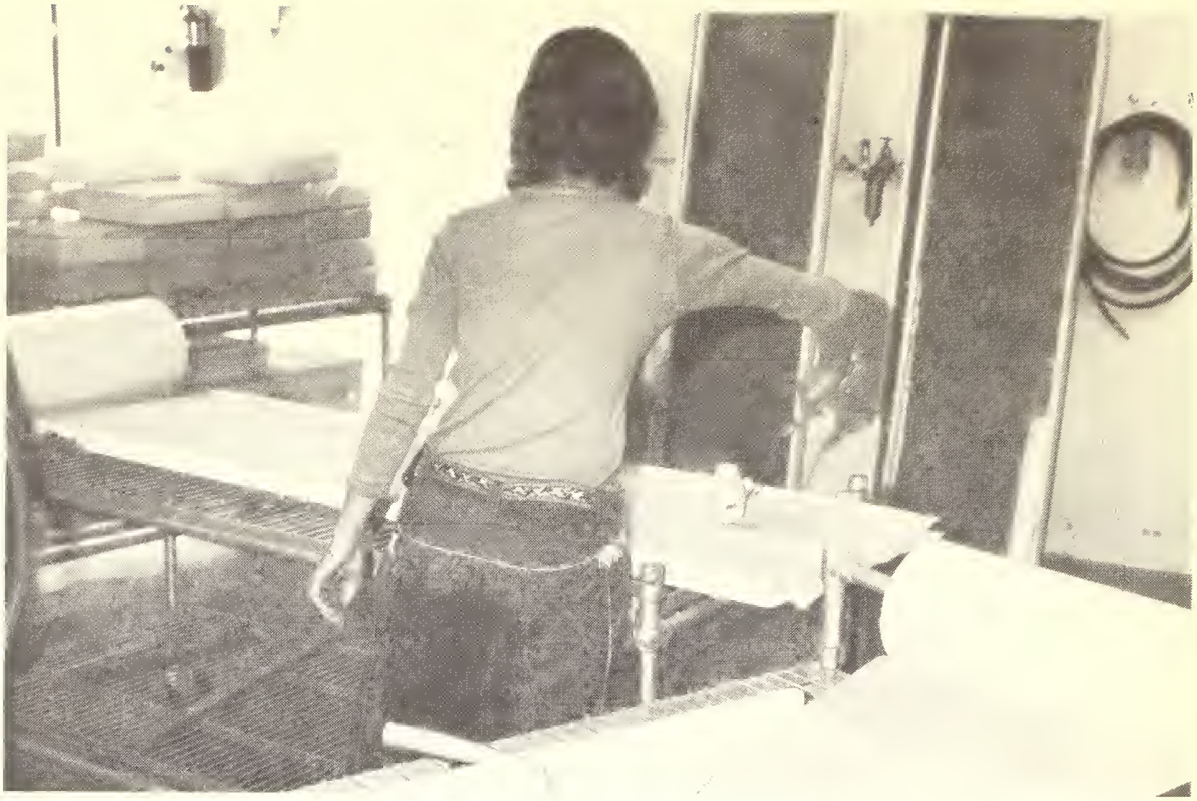

Figure 1.--Spreading hot diet with a smooth paint roller on a diet rack.

plastic. These are notched so as to fit on top of another frame. This arrangement allows successive frames of diet to be stacked on top of each other, and also keeps the racks in alinement with the paper.

After the diet has set and partially cooled (about 1 hour), the 12 layers of diet and paper are rolled lengthwise together into a fairly compact roll approximately 15.9 cm in diameter. Each roll is then cut into 12 slices, 3.8 cm thick. Originally, the cutting was done with a sharp knife, but as production was increased, an electric meat saw was used (fig. 2). As the slices are cut, they are placed on a shelf of a rolling rack[4] and moved into a drying room. The drying room consists of a 6.1- by 6.1- by 2.2-m building with partial block wall 0.9 m high. The rest of the walls and roof are of frame construction and are covered with corrugated opaque fiberglass panels. Outside air is drawn through the building through six 0.5- by 0.5-cm filters by an 0.5-cm exhaust fan. The fan is on a timeclock set to operate for 3-min periods at 15-min intervals. The room is heated with a thermostatically controlled gas heater, which is normally set at 29.4° C, and operates only at night or on cloudy or cool days. On clear days, solar heat keeps the room temperature between 29.4° and 54°, depending on the outside temperature.

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[4] Fye, R. E., Patana, R., Jackson, C. G., and Bryan, D. E. An inexpensive mobile rack for rearing insects. U.S. Dept. Agr., Agr. Res. Serv. ARS 33-138, 3 pp. 1972.

The diet slices are usually cut and placed in the drying room at 2 p.m. and are ready to be put into cartons about 8 a.m. the next morning. The 18-hour drying period was selected after a test was conducted in April 1974 in which fresh slices were weighed and then allowed to dry 3, 6, 12, and 18 hours with the weight loss being 14, 18, 34, and 38 percent, respectively. After pink bollworm larvae had developed in these cartons, the exit holes were counted, and those that had been dried 18 hours had the greatest number of exit holes. This probably is not a true picture of the total number of larvae because a number of larvae could use the same exit hole, but it did indicate an advantage to the 18-hour drying period. If the slices dry much longer than this, the diet becomes too dry and the larvae starve. If the diet slices are too moist, the larvae drown and the excessive moisture favors mold development. In this partial drying process, mainly the top and bottom surfaces of the slices dry out, whereas the diet and paper between these surfaces are still moist. Three slices are placed into each 3.8-liter, glassine-lined paper carton. On top of the diet slices, a piece of Hexcel (fiberglass honeycomb material), 15.2 by 13.3 by 5.1 cm with 1.9-cm-wide cells, is placed as a spacer to totally fill the inside of the carton. The lid is then placed on the carton, and the cartons are stored in the refrigerator until needed. While the cartons are in storage, the moisture equalizes throughout the diet slices.

Eggs are placed daily in the cartons when 3 days of age, and generally hatch after 4 days when held at 28.9° C. The egg sheets are placed on top of the Hexcel with eggs facing the diet. The Hexcel, in addition to filling space in the cartons, also isolates the eggs from the diet. When the eggs were directly

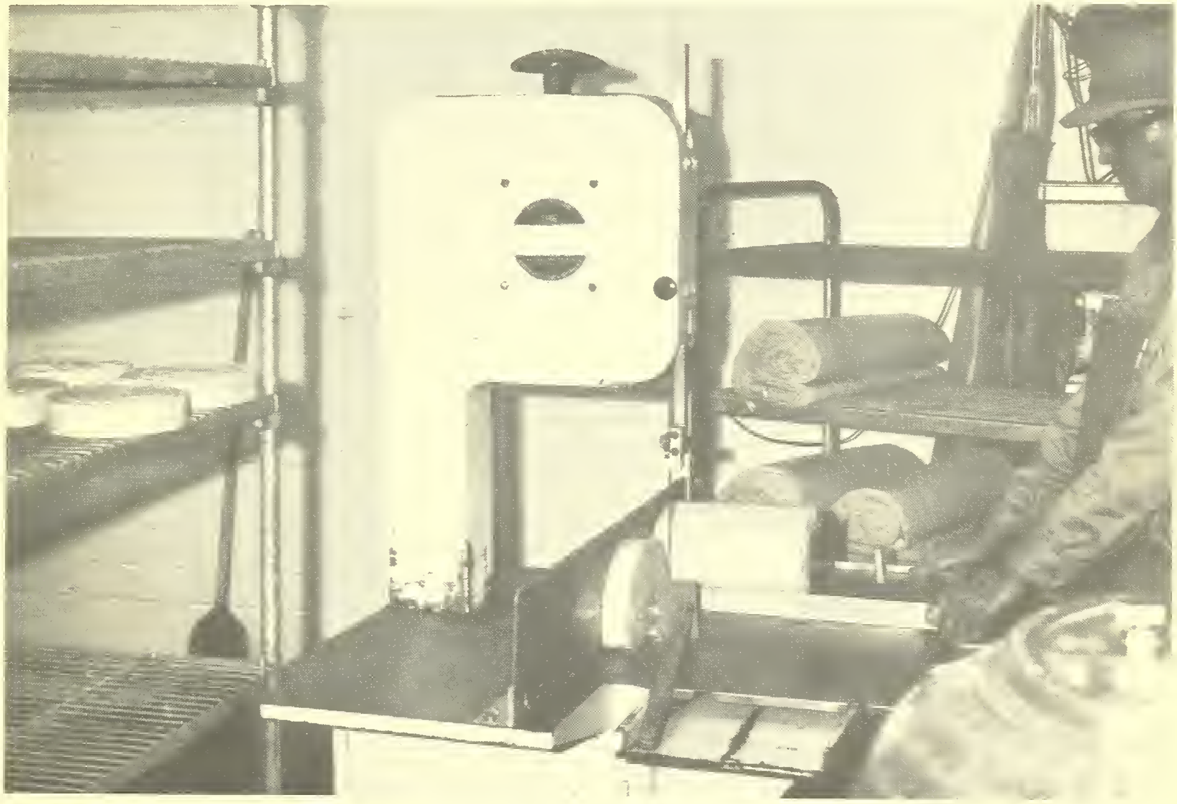

Figure 2.--Cutting diet slices with an electric meat saw.

exposed to the diet surface, they took up moisture, which lowered the percentage of hatch. The egg sheets are covered with a double thickness of facial tissues, which are cut in a 20.3-cm-diameter circle so that when the lid is placed over them the tissue prevents small larvae from escaping, and no exposed tissue is left on the outside of the carton for larvae to pupate in later. After the cartons are closed, they are inverted and placed on a rack. This same procedure was used for rearing the parasite *C. blackburni* Cameron for field release.[5] [6] [7]

These racks have been modified somewhat since they were first used. Rather than use a V-shaped piece of sheet metal with holes to trap and channel larvae,[8] we used two 0.46- by 1.7-m metal sheets held in place with three W-shaped metal supporting straps. The metal sheets fit on each side of the W-strap and allow a 25.4-cm-wide opening the length of the rack between the metal sheets. The sides of the sheets extend beyond the sides of the shelves to catch falling larvae. This open-type arrangement (fig. 3) was developed when it was found that the larvae would congregate in great numbers and injure one another in the bottom of the "V" between the openings.

The larvae begin to chew their way out of the cartons after 12 days when held at 28.9° C. The cartons are disposed of after 21 days, and the Hexcel is reclaimed, washed in strong sodium hypochlorite solution (0.1 to 0.15 percent), and reused. The number of larvae and pupae remaining in the diet did not justify the labor involved in recovering them (less than 5 percent of the total yield per carton). The larvae are caught in four metal pans on the bottom shelf of the rack. The dimensions of the pans are 0.6 by 0.46 m, and 11.4 cm in depth. The larvae form cells and pupate in a 5.1-cm layer of cornmeal in the bottoms of the pans (fig. 3). At low levels of production, these pans are changed once daily. At higher levels of production (20,000 to 30,000 pupae per day), the pans are changed twice daily. After changing, the larvae and cornmeal are allowed to remain undisturbed for 6 days. By this time, the majority of the larvae have pupated. The cornmeal containing the pupae is sifted through window screening (14 by 18 mesh/2.54 cm) to separate the cells and pupae from the excess cornmeal. This leaves only the cornmeal that is attached to the cocoons.

The cocoons are washed the following day in 0.4- by 0.5- by 0.1-m plastic food pans with a 0.05-percent sodium hypochlorite solution. The bottoms of the plastic food pans are cut out and replaced with a 16- by 16-mesh/2.54 cm nylon screen, which is glued in place with hot glue. The screened pan with the cells and pupae is placed inside an unmodified pan. The unmodified pans, in turn, are placed in a large metal pan, 0.6 by 0.7 by 0.08 m with a drain connected to a recirculating pump. The sodium hypchlorite solution is poured into the metal pan and run through the pump up into the screened plastic pan and over the pupae by

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[5] Bryan, D. E., Fye, R. E. Jackson, C. G., and Patana, R. Releases of *Bracon kirkpatricki* (Wilkinson) and *Chelonus blackburni* Cameron for pink bollworm control in Arizona. U.S. Dept. Agr. Prod. Res. Rpt. 150, 22 pp. 1973.

[6] Bryan, D. E., Fye, R. E., Jackson, C. G., and Patana, R. Releases of parasites for suppression of pink bollworms in Arizona. U.S. Dept. Agr., Agr. Res. Serv. ARS W-7, 8 pp. 1973.

[7] Bryan, D. E., Fye, R. E., Jackson, C. G., and Patana, Raymond. Nonchemical control of pink bollworms. U.S. Dept. Agr., Agr. Res. Serv. ARS W-39, 26 pp. 1976.

[8] See footnote 4.

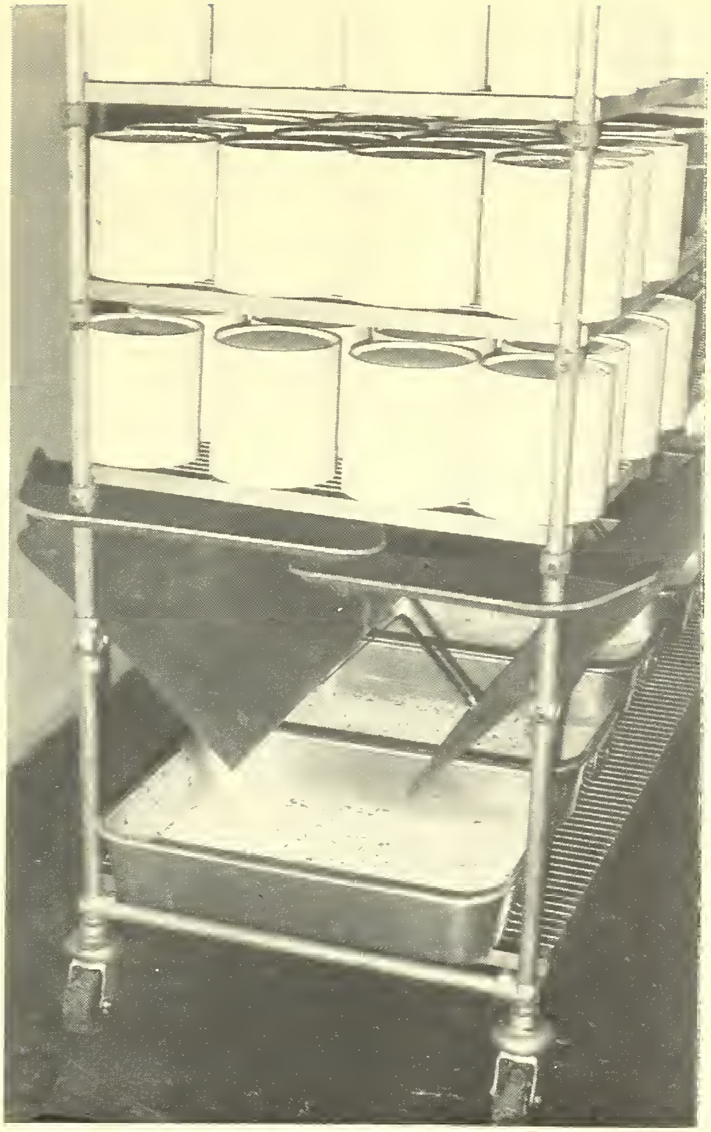

Figure 3.--End view of rack modified to collect pink bollworm larvae cutting out from diet cartons. The larvae are collected in cornmeal in the pans below.

means of a hose. The solution is pumped until it overflows from the bottom plastic pan into the metal pan and back through the pump (fig. 4). The solution is allowed to circulate for approximately 10 to 15 minutes. In this time, the webbings are usually dissolved when 5,000 to 10,000 pupae are washed. When the plastic pans are placed inside one another, there is a 5.1-cm space between the screened bottom pan and the bottom of the unmodified pan. As the pupae are washed, the cornmeal that was attached to the cocoons settles in the space between the pans. After the screened pan with the pupae is removed, the hypochlorite solution is poured off, and the cornmeal is rinsed with water, dried, and reused. The pupae in the screened pan are rinsed with tapwater and allowed to dry.

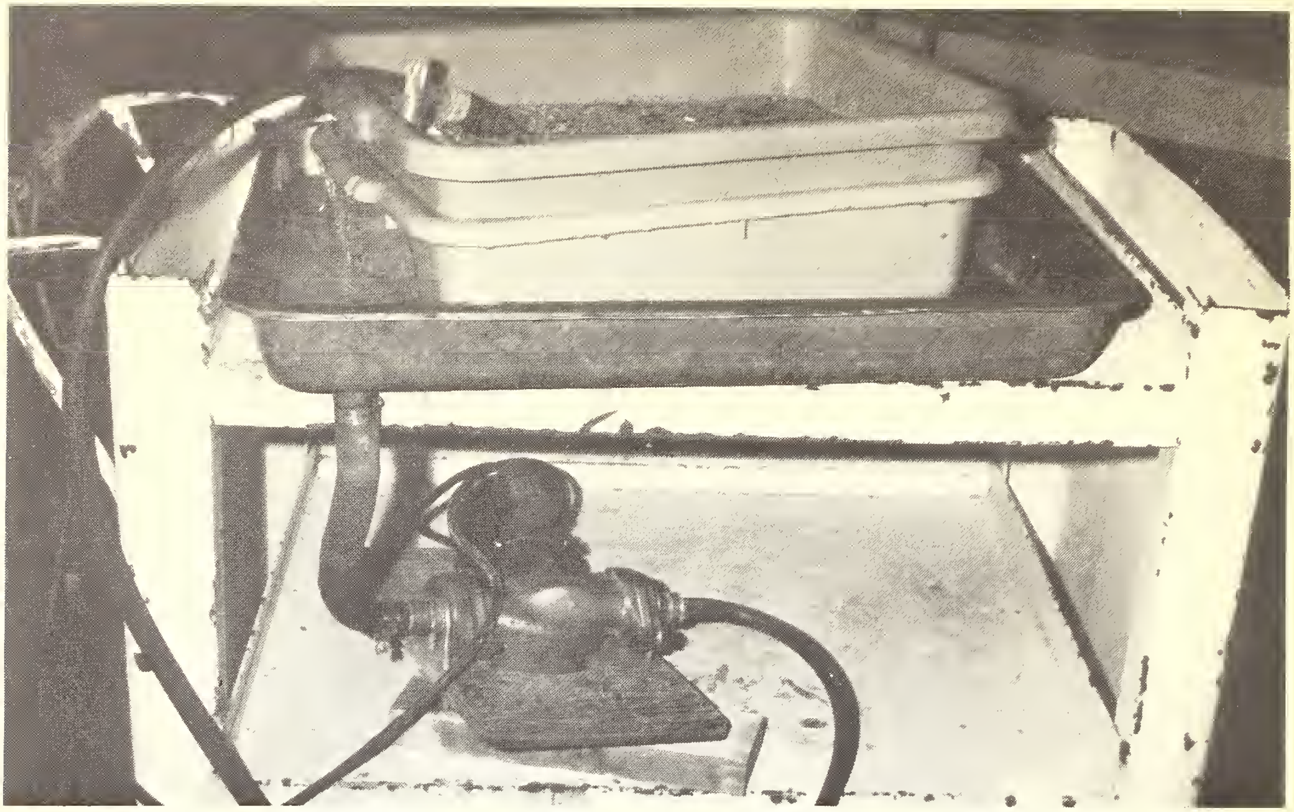

Figure 4.--Dissolving webbing from pink bollworm cocoons with a sodium hypochlorite solution by means of a recirculating pump.

When the pupae are dried, they are measured volumetrically (4 ml = 100) and placed in cages. The cages are made from plastic storage boxes 26.7 by 36.8 by 16.5 cm. The bottoms of the cages are cut out and replaced with a 22.9- by 31.8-cm piece of 14 by 18 mesh/2.54 cm aluminum window screen, which is glued in place with an electric glue gun. A 12.7- by 20.3-cm opening is also cut into the lids and replaced with screen. Approximately 3,000 pupae are put into each cage, the lid is taped on, and the cage and lid are inverted and placed on pans on a rack with bottom side up (fig. 5). When the moths begin to emerge (approximately 2 to 3 days), a cotton pad, 5.1 by 7.6 cm, is placed on one end of the cage and moistened with a 10-percent sugar solution. The pads are changed every other day and are moistened on the day in between.

About 2 days after emergence, a plastic screen of the same mesh is placed over the cage screen. On top of this, a sheet of 20.3- by 26.7-cm mimeograph paper is placed and weighted to fit tightly against the screens. The moths oviposit up through the double layer of screen and onto the paper. Previously, 11-cm filter papers were used,[9] but with increased production, it was found that besides being more expensive, they required excessive handling, particularly in handling eggs for parasitism by *Chelonus blackburni*. By using the large sheets, it is easier to change the oviposition cages, and also to expose the eggs to the

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[9] See footnote 5.

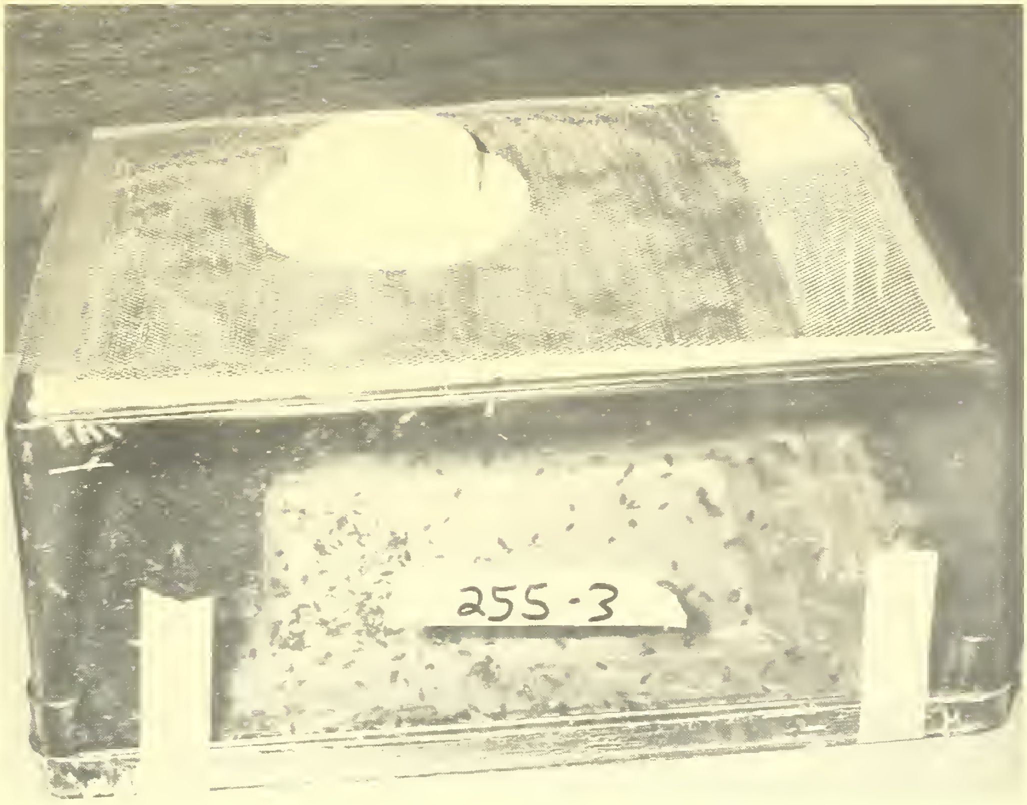

Figure 5.--Pink bollworm moth cage with newly emerged moths, watering pad, additional plastic screen, and weighted filter paper.

wasps. The mimeograph paper sheets with the eggs are cut into quarters for later infestation. The egg sheets that are to be used for the parent culture are surface-sterilized with a 0.02-percent formaldhyde solution, rinsed with tapwater, and dried. The egg sheets used for parasitization are not surface sterilized.

RESULTS AND DISCUSSION

In 1971, counts were made of eggs from the 11-cm filter papers. The more heavily laden filter papers used for maintaining the parent stock yielded an average of 1,500 eggs per filter paper, and the average count for lighter papers was 800 eggs per filter paper. Counts were also made of the mimeograph sheets, which averaged 4,000 eggs per sheet. During the summer of 1971, two of the heavily egg-laden filter papers were used per carton of diet (averaging 3,000 eggs per carton). During August 1971, for a period of 5 days, a diet carton was implanted daily with 3,000 eggs. Cutout larvae from these cartons were counted daily after 14 days.

At the end of 21 days, the cartons were opened, and the remaining larvae and pupae were counted. At 3,000 eggs per carton, a 16-percent return was realized, yielding an average of 490 pupae per carton. Since the main objective was to produce as many pupae as possible, the 3,000 eggs-per-carton figure was chosen.

The low percentage of return was thought to be due to three main factors: (1) Some newly hatched larvae escaped from the cartons around the seams of the bottoms when inverted; (2) the diet was too tightly rolled and not sufficiently dried out, thereby drowning small larvae; and (3) some loss was due to cannibalism both inside and outside the carton.

During the 1971 season, 283,000 *C. blackburni* were produced for field release.[10] At that time, one 11.4-cm section of diet roll per carton was being used. If the diameter of this diet section was too small, the hatching larvae followed the side of the inverted carton up and out through the seams in the bottom of the carton. If the section of diet was too large, it made the diet surface so tight that the larvae would be unable to penetrate up in between the layers of diet. One way of overcoming these problems with size variability of the diet sections was to cut them into thinner slices. In 1972, the diet sections were cut into 5.7-cm slices, and two of the slices were placed in the cartons. This method allowed the slices to be spread out so that the sides of the slices touched the interior sides of the carton and forced most of the larvae into the diet so they could start feeding. At this same time, the number of eggs placed into each carton was increased to about 4,000 eggs per carton. During the 1972 season, 3.1 million *C. blackburni* reared by this procedure were released in the field.[11]

In 1973, the thickness of the slices was reduced to 3.8 cm with three slices used per carton, and the number of eggs was increased to approximately 5,000 per carton. This increase resulted in an increased pupal production per carton until about mid-May with the average number of pupae per carton being well above 2,000. After mid-May, production began to drop off. At first, the production drop was thought to be the result of a previous air-conditioning failure when adults and caged pupae had been exposed to temperatures of 35° C. for about a 6-hour period. However, production continued to drop and reached a low in mid-August. During this same period, developed larvae were collected in pans of Wheast, and a new shipment of the material was found to be of a much finer texture than that which had been used previously. The older Wheast had a somewhat flaky consistence, whereas the new material was rather powdery. The "cut-out" larvae would not burrow into the Wheast to cell up in the new material as they had done previously. With the change in consistency, there had also been a change in formulation. The ash (salt) content of the Wheast had been raised from 10 to 20 percent. The fact that Wheast was being used in the bean diet as a substitute for Brewer's yeast provided an additional explanation for the lowered production. Brewer's yeast was returned to the diet and production increased. Cornmeal was substituted for Wheast as a pupation medium.

In 1974, the number of eggs per carton was increased to 6,000, resulting in a higher average number of pupae per carton. Production figures covering an 11-week summer period for 4 years are given in table 1.

In table 1, a marked increase in production can be seen between 1971 and 1972 and also in the following years. On a weekly basis, the average number of pupae per carton per day increased two- to fivefold between 1971 and 1972. The 1972 increase can probably be attributed to the 5.7-cm diet slices and the increased number of eggs. The drop in production seen in August 1973 was due to

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[10] See footnote 5.

[11] See footnote 6.

TABLE 1.--*Four-year pink bollworm production for an 11-week period, June 21 through August 30, listing total weekly production and average number of pupae per carton per day*

Week of--	1971		1972		1973		1974	
	Total pupae per week	Average carton per day[1]	Total pupae per week	Average carton per day[1]	Total pupae per week	Average carton per day[1]	Total pupae per week	Average carton per day[1]
Number eggs per carton	3,000		4,000		5,000		6,000	
June 21	41,000	398	233,750	2,087	107,250	1,094	239,000	2,134
28	27,000	257	157,350	1,404	131,850	897	226,000	2,018
July 5	34,000	324	125,500	1,120	131,350	894	173,750	1,551
12	70,700	673	140,600	1,255	89,000	605	181,325	1,619
19	40,750	388	173,800	1,551	72,500	493	169,750	1,516
26	48,950	466	94,250	842	61,250	417	164,875	1,472
Aug. 2	57,000	543	131,000	1,169	58,750	400	205,000	1,830
9	59,300	565	149,150	1,332	42,250	287	163,250	1,943
16	55,700	531	144,500	1,290	38,450	392	85,500	2,035
23	66,875	637	213,000	1,902	53,050	541	69,250	2,473
30	42,750	407	133,500	1,192	47,750	487	60,000	2,143

[1] To find the number of cartons used per day, divide the weekly total by the average carton per day and divide the quotient by 7. Generally, about 16 cartons of diet were used per day except where the number of cartons was raised to offset drops in production.

the formulation change in the Wheast. During the 1974 season, the rearing was stabilized, and there were only minor weekly fluctuations in production, thus allowing more stabilized production and improved parasite production for field release. During the 1974 season,[12] 4.8 million *C. blackburni* were released in the field. Production was intentionally reduced in August of each year except for 1973. Pink bollworm eggs parasitized before that time provided the parasites for the final field releases, and eggs parasitized after August were used to maintain the parasite culture.

The 3.8-liter carton has been used primarily for laboratory rearing and parasite field release, but the rolled, layered diet has been used successfully in 1.9- and 0.47-liter cartons for laboratory studies.

The diet and its preparation could easily be adapted to smaller scale programs and possibly to other insects.

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[12] See footnote 7.

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